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LIQUID AND GAS CHROMATOGRAPHIC DETERMINATION
OF 1,3,5-TRINITROBENZENE IN WATER

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Two methods are described for the analysis of 1,3,5-trinitrobenzene in well water used in aquatic toxicity testing. One method by HPLC and another by GC are covered in detail. The precision and accuracy of both methods are presented along with several advantages and disadvantages of each method.

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FIGURES
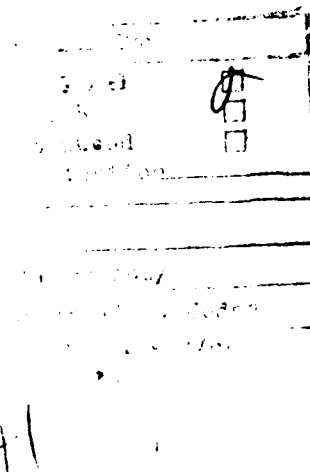
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TABLES

INTRODUCTION AND OBJECTIVES

Investigations conducted at the U.S. Army Medical Bioengineering Research and Development Laboratory (Aquatic Toxicology Section) have shown that 1,3,5-trinitrobenzene (TNB) is toxic to freshwater aquatic organisms. Further investigations are now being performed by the Aquatic Toxicology Section to determine the relative effects of TNB in constant and fluctuating applications on the water flea Daphia magna.

In support of the Aquatic Toxicology Section, ~~it was our~~ *this report pertains to a* task to develop chromatographic methods for the determination of TNB in water. The relative water solubility and available UV chromophore of TNB make it a suitable candidate for direct injection reversed phase high performance liquid chromatography (RP-HPLC). Furthermore, TNB's chemical and physical properties also make it an attractive choice for gas chromatography (GC) employing a nitrogen phosphorus detector (NPD).

The liquid and gas chromatographic methods reported herein are intended to be quick, sensitive, and reproducible for the determination of TNB in water.

METHODS AND MATERIALS

ANALYTICAL INSTRUMENTATION

A Waters liquid chromatographic system (Waters Assoc., Milford, MA) was employed throughout the study. The system consisted of the following components: a model M6000A solvent delivery system, a model 721 programmable system controller, a model 730 data module, and a model 710B WISP Auto-sampler. The detector was a Spectroflow 783 programmable absorbance detector (Kratos Analytical Instruments, Ramsey, NJ).

A Hewlett Packard Model 5830A gas chromatograph equipped with NPD and a model 7671A automatic sampler (Hewlett Packard Corp., Avondale, PA) were used to perform the GC analyses. In addition, the gas chromatograph was equipped with a General Electric model l5EHG1B1 hydrogen generator (General Electric, Aircraft Equipment Division, Wilmington, MA).

CHEMICALS

The methanol used was "HPLC grade" from Burdick and Jackson Laboratories (Muskegon, MI). Reagent grade water was obtained with a Milli-Q System (Millipore, Bedford, MA) and had a resistance of 18 megohms-cm. Pesticide grade acetone and toluene was purchased from Fisher Scientific Corp. (Pittsburgh, PA).

Dursban (98.6% pure) was obtained (Chem Service, Inc., West Chester, PA) and was used without further purification.

The 1,3,5-trinitrobenzene (99+% pure) used throughout this study was synthesized and recrystallized in-house.[2]

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HPLC CONDITIONS

Separation was achieved by using a Supelcosil LC-18 column (5 micron particle size, 25 cm x 4.6 mm I.D., Supelco Inc., Bellefonte, PA). The mobile phase consisted of 60 percent methanol in water at a flow rate of 1.2 mL/min. The column effluent was monitored spectrophotometrically at 254 nm, 0.05 absorbance units full scale (AUFS) for TNB concentrations above 0.10 mg/L. For TNB concentrations below 0.10 mg/L, the detector sensitivity was adjusted to 0.005 AUFS. The injection volume was always 100 µL.

GAS CHROMATOGRAPHIC CONDITIONS

Separation was performed on a glass column (6 ft x 1/4 in O.D., and 2 mm I.D.) packed with 3 percent OV-1 on 80/100 mesh Gas Chrom Q (Supelco, Inc., Bellefonte, PA).

Helium (99.997%) was used as a carrier gas at a flow rate of 30 mL/min. Air (zero grade) and hydrogen (99.95%) functioned as the detector gases with flow rates of 50 and 3 mL/min, respectfully. The oven temperature was operated isothermally at a temperature of $220^{\circ}C$. The injection port and detector temperatures were 250° and $300^{\circ}C$, respectively. The injection volume of the autosampler was 1 µL throughout this study.

PREPARATION OF STOCK AND STANDARD SOLUTIONS

HPLC Solutions

A low-level stock solution containing TNB was prepared by dissolving the solute in methanol to a concentration of 5 mg/L. Aliquots of the stock solution were diluted with reagent grade water to yield TNB standards with concentrations ranging from 0.010 mg/L to 0.100 mg/L.

For high concentration analysis, two separate stock solutions of TNB were prepared by dissolving 3 mg and 5 mg of TNB in 100 mL of methanol to yield stock solution concentrations of 30 mg/L and 50 mg/L, respectively.

Aliquots of the stock solutions were diluted to 100 mL in reagent grade water to prepare TNB standards with concentrations ranging from 0.30 mg/L to 10.00 mg/L.

All stock and standard solutions were prepared fresh the day of the analysis.

Gas Chromatographic Solutions

The TNB stock solution was prepared by dissolving 5.51 mg of pure TNB (99.9+%) in 50 mL of acetone to give a final concentration of 110 mg/L. Aliquots of the stock solution were diluted with reagent grade water to yield TNB standard solutions with concentrations ranging from 0.022 mg/L to 1.102 mg/L. All stocks were prepared fresh the day of analysis.

The extraction solvent was prepared by dissolving 7.3 mg of dursban (internal standard) into 100 mL of toluene. This solution was diluted with

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toluene to obtain a final concentration of 0.73 mg/L. This solution was used throughout the test period.

SAMPLE PREPARATION AND HANDLING

For HPLC analysis, each water sample containing a concentration in excess of 10 mg/L TNB was diluted with reagent grade water to obtain a sample concentration within the upper and lower limits of the appropriate standard curve. No further sample preparation was required; each sample and standard was injected a minimum of four times.

GC analysis required that 10-mL aliquots of samples and working standards be placed into acid-washed and acetone-washed 15-mL glass vials. One milliliter of toluene stock solution, spiked with dursban as an internal standard, was added to each sample and standard solution. All vials were capped tightly and shaken vigorously by hand for 2 minutes and allowed to stand for 5 minutes to allow the water/toluene layers to separate. Approximately 1.5 mL of the toluene layer (top) was removed with a Pasteur pipet and placed in a 2 mL autosampler glass vial for analysis.

<div align="center">RESULTS</div>

HPLC METHOD

Figure 1 shows HPLC chromatograms obtained from a water blank and a water sample containing 0.020 mg/L TNB with a retention time of 5.85 minutes. The TNB peak is symmetrical and well separated from other peaks in the chromatogram.

Calibration curves were constructed by plotting peak areas for all working standards against their concentration. Standard curve data for the HPLC analysis is presented in Table 1. Precision was determined by injecting a low and high concentration level sample four times on three separate days. The mean, standard deviation and relative standard deviation are given in Table 2. The relative standard deviation for precision was less than or equal to 3.16 percent for all concentration levels.

In order to evaluate accuracy, recovery studies were conducted on well water samples spiked with TNB. This was accomplished by taking sample aliquots at four concentration levels and adding a known volume of the appropriate TNB standard to each sample. Each aliquot was then analyzed four times to obtain a mean, standard deviation, relative standard deviation, and percent recovery (Table 3). The recovery of TNB in water was greater than or equal to 93.89 percent for all concentration levels.

GC METHOD

Figure 4 shows a routine chromatogram of TNB and dursban (internal standard) with retention times of 1.15 and 2.69 minutes, respectively. Peak shape of TNB and internal standard (Dursban) and their separation, although good, could be improved by reducing the column oven temperature. This lower

oven temperature would increase the running time with negligible improvement in accuracy or precision, while decreasing sensitivity. In our application, we were more concerned with sample turnaround time, precision and accuracy than peak shape.

A TNB calibration curve was constructed from the peak area ratios of TNB to the internal standard (dursban) vs. the concentration of TNB in each standard (Figure 5). From Figure 5 it can be seen that the calibration is linear over the concentration range of 0.022 mg/L to 1.102 mg/L used throughout this study. The values of TNB's concentration were determined by regression analysis. Standard curve data are presented in Table 4.

Precision of the method was determined by analyzing the same sample five times on four different days to determine the mean, standard deviation, and relative standard deviation (Table 5). This was done for a low and a high concentration. The average relative standard deviation for the high concentration was 2.30 percent and 4.61 percent for the low concentration. The limit of detection was defined here as the lowest concentration that could be reproduced five times with a relative standard deviation of not more than 10 percent. For this method, the detection limit was determined to be 0.020 mg/L.

Percent recovery or accuracy (Table 6) was determined by spiking a low and a high concentration sample. Five 9-mL aliquots, each, were removed from the low and the high concentration sample. A 1-mL spike of concentrated TNB solution was added to each low concentration aliquot to double the concentration. A 1-ml spike of concentrated TNB solution was added to each high concentration aliquot to bring the concentration up to approximately 75 percent of the upper limit of the calibration range. Each of the samples was analyzed to obtain a mean, percent recovery, standard deviation, and relative standard deviation. This method at the higher concentrations had an average percent recovery of 97.4 while at lower concentration the average percent recovery dropped to 92.1. While the accuracy for this method was less than that for HPLC, this can be explained by the wide concentration range of the calibration curve.

DISCUSSION

The chromatographic running time of the HPLC method is twice as long as the GC method. However, this is of little significance when compared to the shorter sample preparation time of the HPLC method. The HPLC method requires almost no sample preparation in analyses where relatively clean water samples can be quickly filtered and injected directly on the HPLC. The saving in sample preparation time for the HPLC method decreases rapidly as the sample matrix becomes more complex and requires more than a simple sample filtration before injection. This method appears slightly more precise and accurate than the GC method, but this could be due in part to the use of two standard curves for a concentration range where the GC method employs only one. In addition, the absence of an extraction step in this method may account for some of its enhanced precision and accuracy over the GC method.

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CONCLUSION

The HPLC method described is suitably accurate, rapid, and sensitive for determining TNB in relatively clean well water at a wide range of concentration levels. The GC method is also sensitive and accurate, but requires slightly more sample preparation than the HPLC method.

The two methods presented here for the analysis of TNB in water are both precise and accurate. There are, however, advantages and disadvantages to each that must be considered before one decides on the method of choice for a given type of sample. The analyst must look at essential factors, such as sample matrix, required precision and accuracy, sample turnaround time, and available instrumentation before deciding on one method.
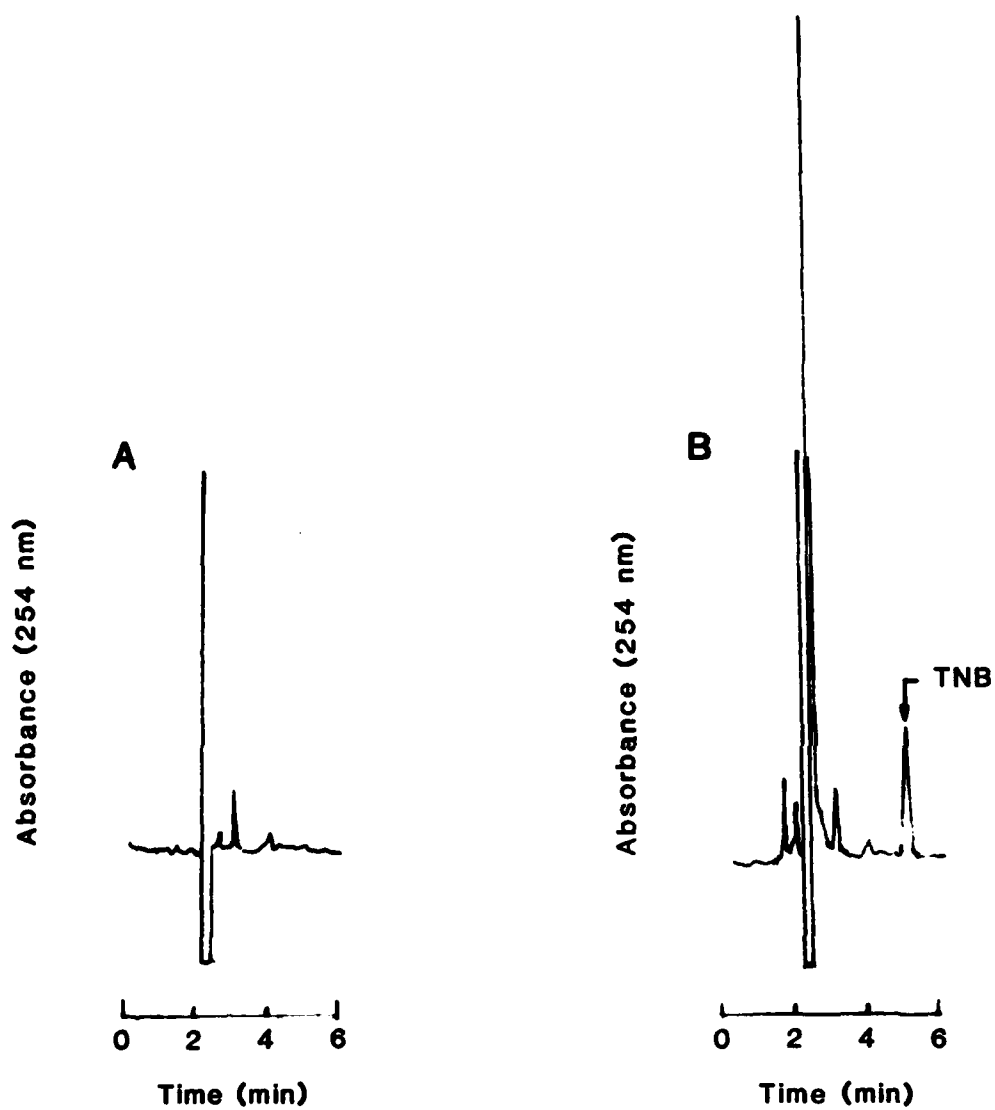
Figure 1. Typical HPLC chromatogram of a (A) blank and (B) 0.020 mg/L TNB water sample.
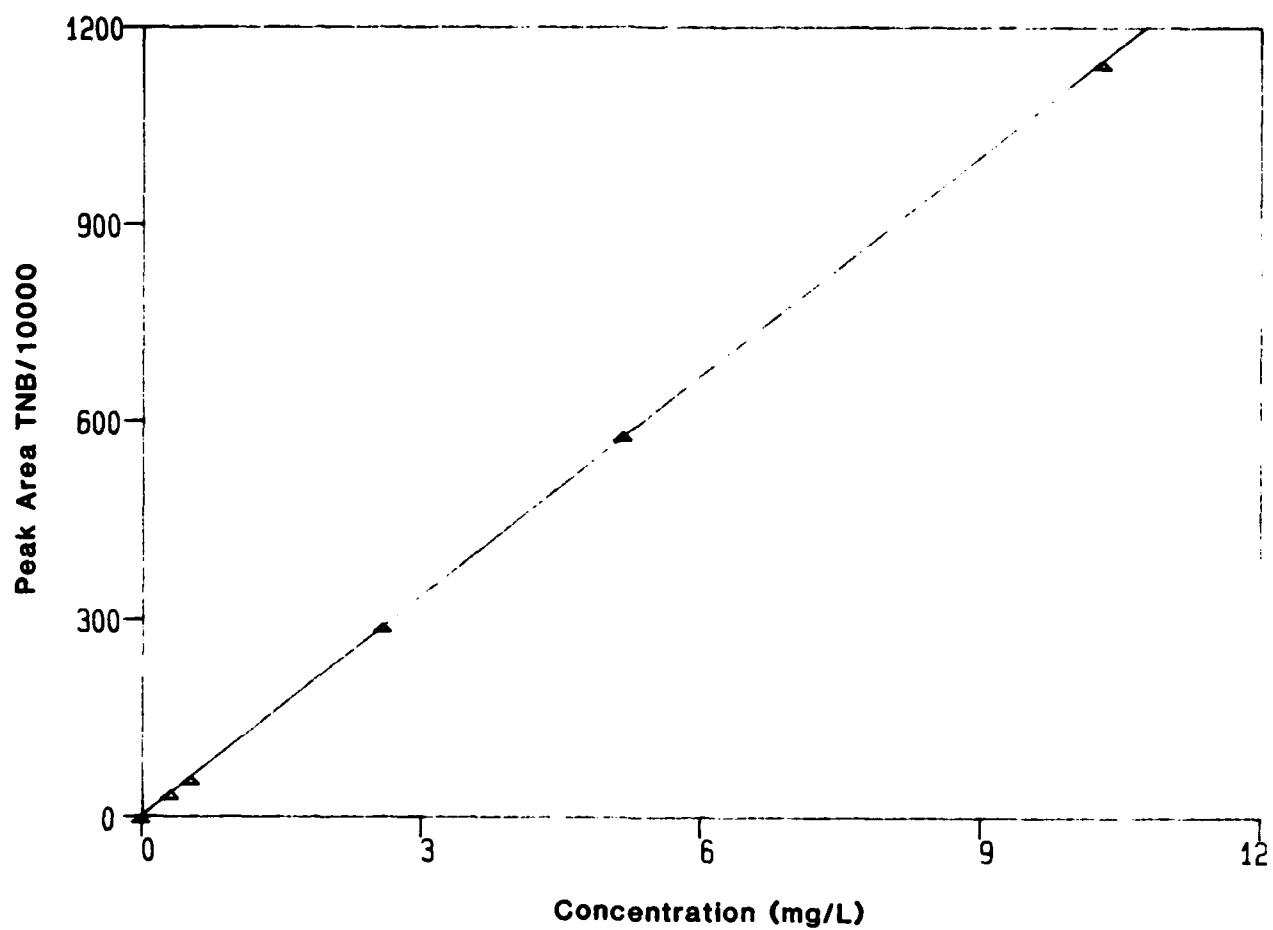
Figure 2. HPLC high level standard curve for TNB.
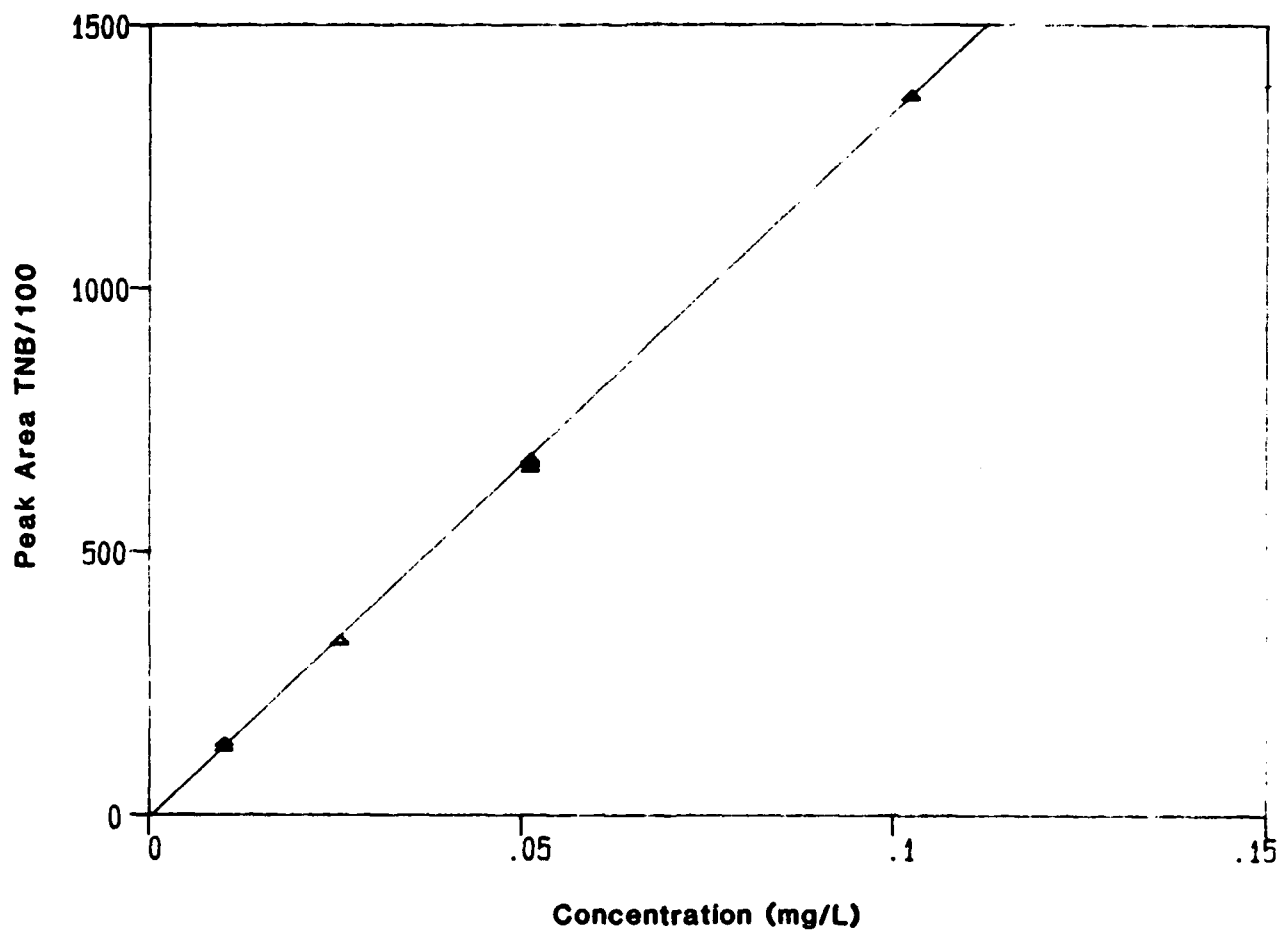
Figure 3. HPLC low level standard curve for TNB.
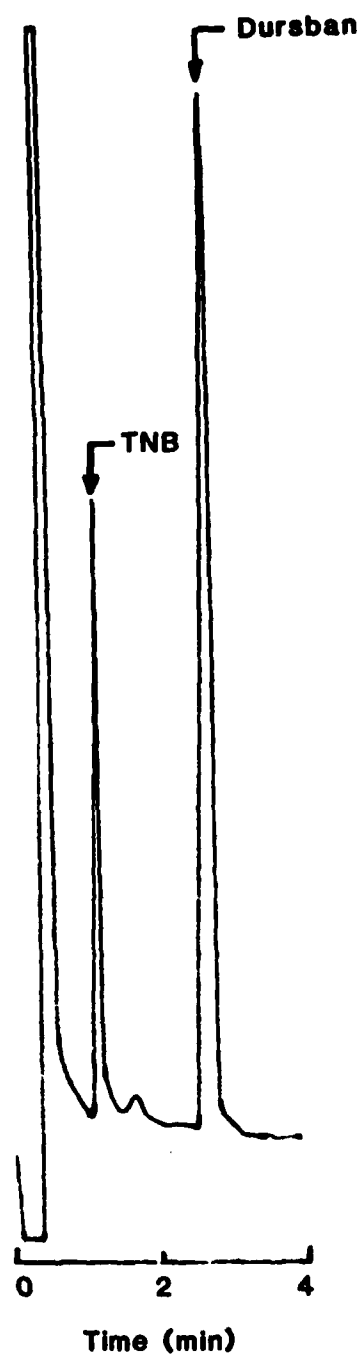
Figure 4. Gas chromatogram showing separation of TNB and dursban. Condition: 6′, 3% OV-1 on Gas Chrom Q, 100-200 mesh, 220°C isothermal.

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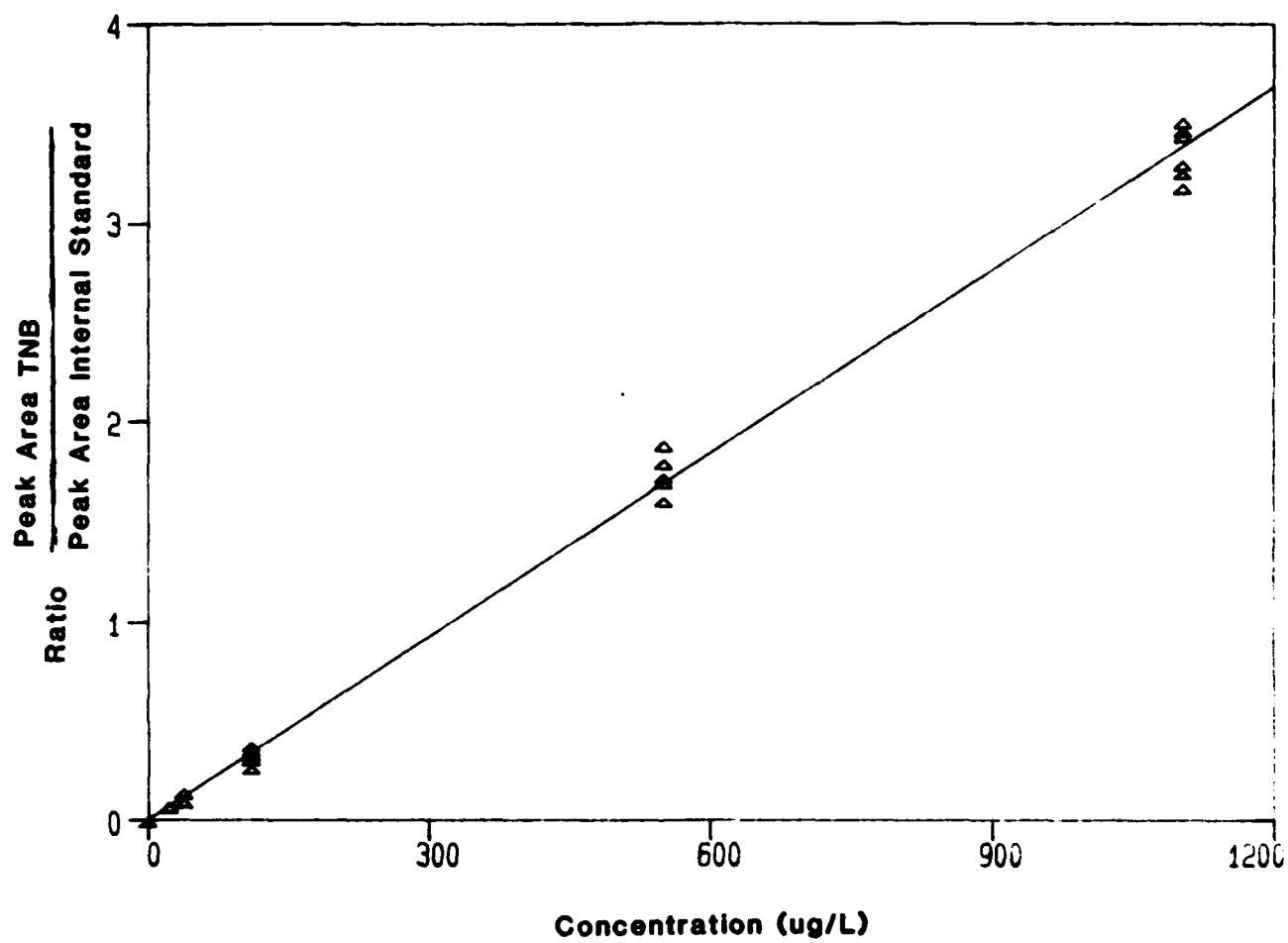
Figure 5. Plot of GC standard curve for TNB.

TABLE 1. LOW AND HIGH CONCENTRATION TNB STANDARD CURVE DATA (HPLC)

Day	Slope	R^2 Value	Y-Intercept
	Low Concentration		
1	1,335,883	1.0000	−463
2	1,320,123	0.9999	−286
3	1,318,389	1.0000	−93
	High Concentration		
1	1,081,692	0.9999	20,499
2	1,112,253	1.0000	13,244
3	1,112,559	1.0000	14,660

TABLE 2. TNB PRECISION DATA (HPLC)

Day	Mean (mg/L)	s.d.	RSD (%)
	Low Concentration		
1	0.010	0.0002	1.92
2	0.010	0.0002	2.08
3	0.010	0.0003	3.16
	High Concentration		
1	9.43	0.01	0.11
2	9.21	0.01	0.11
3	9.20	0.01	0.11

TABLE 3. ACCURACY DATA (HPLC)

Day	Mean (mg/L)	s.d.	RSD (%)	% Recovery
Concentration 1				
1	0.020	0.0002	1.03	100.52
2	0.019	0.0003	1.55	99.48
3	0.020	0.0005	2.55	101.03
Concentration 2				
1	0.097	0.0001	0.10	99.90
2	0.098	0.0005	0.51	101.64
3	0.099	0.0008	0.81	101.64
Concentration 3				
1	5.64	0.01	0.18	101.99
2	5.27	0.01	0.19	98.50
3	4.83	0.01	0.21	99.59
Concentration 4				
1	9.42	0.01	0.11	96.32
2	8.92	0.01	0.11	93.89
3	8.54	0.01	0.12	94.89

TABLE 4. STANDARD CURVE DATA (GC)

Day	Slope	R -Value	Y-Intercept
1	0.0031	0.9971	0.0047
2	0.0052	0.9947	-0.0057
3	0.0047	0.9959	-0.0047
4	0.0055	0.9987	-0.0294

TABLE 5. TNB PRECISION DATA (GC)

Day	Mean (mg/L)	s.d.	RSD (%)
	Low Concentration		
1	0.018	0.0004	2.22
2	0.017	0.0019	11.18
3	0.019	0.0003	1.58
4	0.023	0.0008	3.48
	High Concentration		
1	1.082	0.009	0.83
2	1.032	0.024	2.32
3	1.032	0.014	1.36
4	1.112	0.052	4.68

TABLE 6. ACCURACY DATA (GC)

Day	Mean (mg/L)	s.d.	RSD (%)	% Recovery
	Low Concentration			
1	0.042	0.0005	1.19	101.5
2	0.033	0.0004	1.21	83.1
3	0.035	0.0010	2.86	87.1
4	0.042	0.0009	2.14	96.6
	High Concentration			
1	0.725	0.017	2.34	101.2
2	0.629	0.014	2.23	93.0
3	0.678	0.009	1.33	99.3
4	0.710	0.014	1.97	96.2

LITERATURE CITED

1. van der Schalie, W. H. 1983. The Acute and Chronic Toxicity of 3,5-Dinitroaniline, 1,3-Dinitrobenzene, and 1,3,5-Trinitrobenzene to Freshwater Aquatic Organisms. Technical Report 8305, AD A138408. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD.

2. Gilman, H., ed. 1944. Organic Syntheses, Collective Volume I, pp. 541-544. John Wiley & Sons, New York.

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